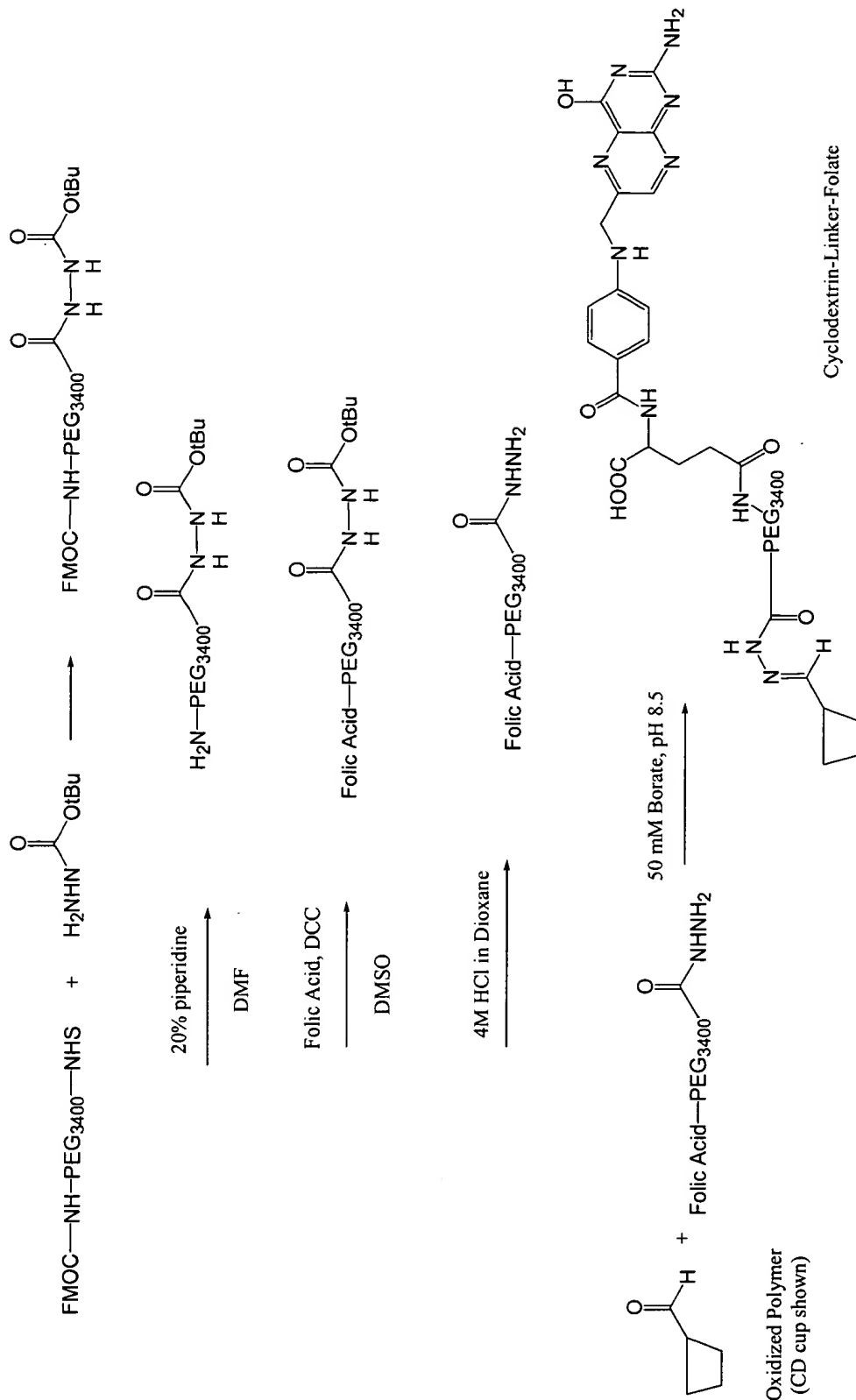
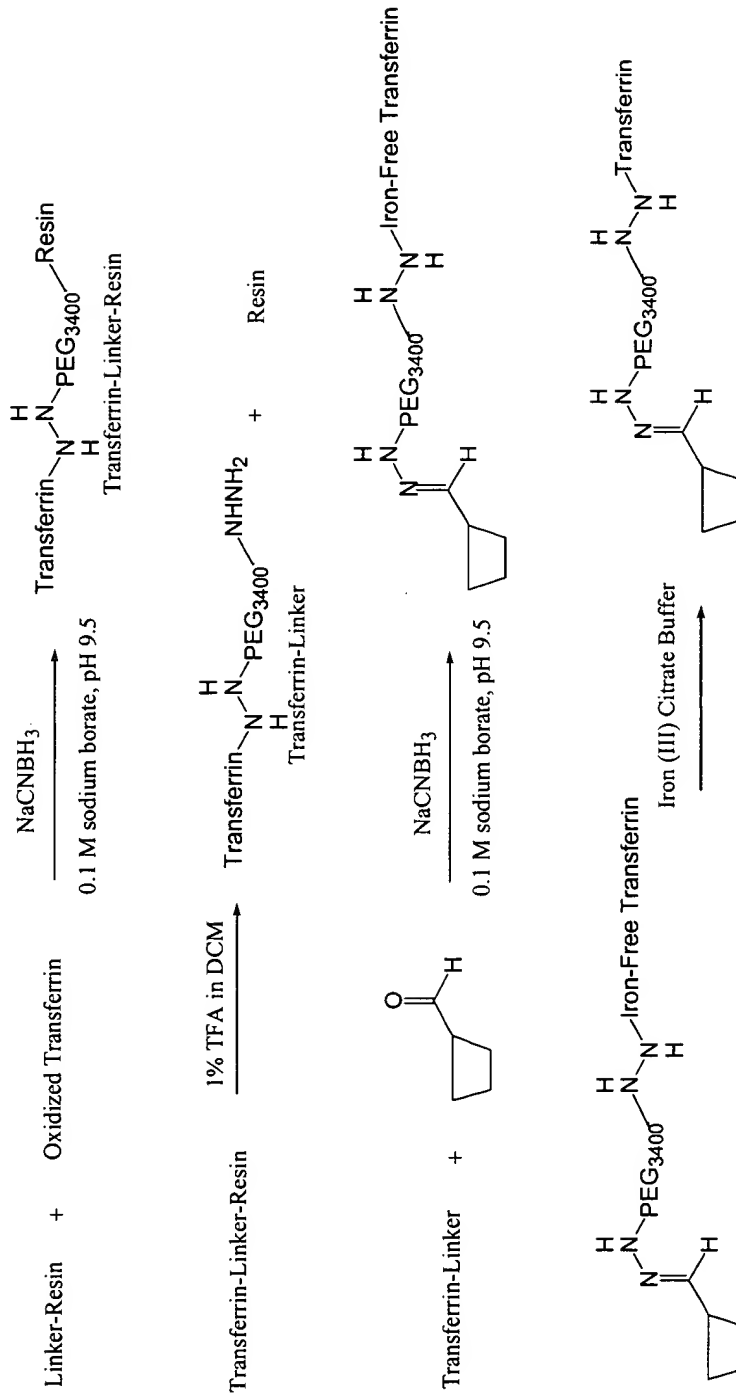


SYNTHESIS OF FOLIC ACID-PEG-HYDRAZIDE



TRANSFERRIN ATTACHMENT TO CYCLODEXTRIN POLYMER



58	3
59	4A
60	4B

Appendix B contains clean copies of Figures 1-4 with drawing labels.

- ✓
5. On page 52, delete the paragraph starting at line 8, and insert the following paragraph:
-

BHK-21 cells were plated in 24 well plates at a cell density of 60,000 cells/well 24 hours before transfection. Plasmids encoding the luciferase gene were encapsulated by the CD-polymer as in Example 23 except copolymer 15 was replaced with copolymer 16 and that the DNA/polymer complexes successfully transfected BHK-21 cells at charge ratios of 10, 20, 30, and 40 with maximum transfection at polymer amine:DNA phosphate charge ratio of 20. Media solution containing the DNA/polymer complexes was added to cultured cells and replaced with fresh media after 24 hours of incubation at 37°C. The cells were lysed 48 hours after transfection. Appropriate substrates for the luciferase light assay were added to the cell lysate. Luciferase activity, measured in terms of light units produced, was quantified by a luminometer. The results are illustrated below. DNA/polymer complexes successfully transfected BHK-21 cells at a charge ratios of 6, 12, and 24. Cell lysate was also used to determine cell viability by the Lowry protein assay. (Lowry et al., *Journal of Biological Chemistry*, Vol. 193, 265-275 (1951)). The results are illustrated in Figures 1A and 1B. Maximum toxicity was seen at a polymer amine: DNA phosphate charge ratios of 40 and 50 with 33% cell survival.

- ✓
6. On page 55, please delete the paragraph starting at line 15 and insert the following paragraph:
-

F8

Plasmids encoding the luciferase gene were encapsulated by the CD-polymer as in Example 23 except copolymer 15 was replaced with copolymer 16. The DNA/polymer complexes were used to successfully transfect BHK-21 or CHO-K1 cells, each plated in 24 well plates at a cell density of 60,000 cells/well 24 hours before transfection, at various charge ratios in 10% serum and serum-free conditions following the procedure outlined in Example 27. The cells were lysed 48 hours after transfection. Appropriate substrates for the luciferase light assay were added to the cell lysate. Luciferase activity, measured in terms of light units produced (*i.e.*, relative light units (RLU)), was quantified by a luminometer. Cell lysate was also used to determine cell viability by the Lowry protein assay. (Lowry et al., *Journal of Biological Chemistry*, Vol. 193, 265-275 (1951)). Toxicity was measured by determining total cellular protein in the wells 48 hours after transfection. The transfection and cell survival results in 10% serum and serum free media are illustrated in Figures 2 and 3.

- ✓
7. On page 59, please delete the paragraph starting at line 3, and insert the following paragraph:
-

F9

Following the procedure of Example 32, transfection efficiency and toxicity of various non-viral vectors with BHK-21 and CHO-K1 cells were studied and compared against those

F9
concluded

achieved with DNA/copolymer 16 complexes. The BHK-21 and CHO-K1 cells were transfected at a range of charge ratios and starting cell densities for all vectors in serum-free media. The results are illustrated in Figures 4A and 4B, and illustrate the optimum transfection conditions found for each vector.

8. At age 7, line 20, please insert the following description of the figures before "Detailed Description of the Invention,":
-

Brief Description of the Figures:

Figure 1 depicts Transfection Studies with Plasmids Encoding *Luciferase Reporting Gene*:

Figure 1A, Transfection with copolymer 16; and Figure 1B, Toxicity of copolymer 16 to BHK-21.

F10

Figure 2 depicts Transfection and Toxicity of Copoloymer 16 to BHK-21.

Figure 3 depicts Transfection and Toxicity of Copolymer 16 to CHO-K1.

Figure 4 relates to Comparative Example 1 and depicts Transfection Studies with Plasmids

Encoding *Luciferase Reporter Gene*: Figure 4A, Relative Light Units; and Figure 4B, Fraction Cell Survival
